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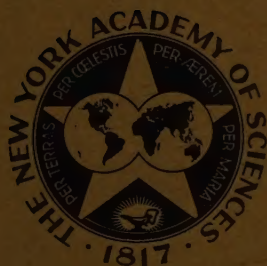
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AN EXPERIMENTAL ANALYSIS OF THE
HYPOTHALAMIC-HYPOPHYSIAL-THYROID
RELATIONSHIP IN THE RAT

By

SAVINO A. D'ANGELO AND RONALD E. TRAUM



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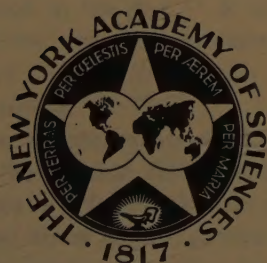
AN EXPERIMENTAL ANALYSIS OF THE
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RELATIONSHIP IN THE RAT

By

SAVINO A. D'ANGELO AND RONALD E. TRAUM

*The Daniel Baugh Institute of Anatomy,
Jefferson Medical College, Philadelphia, Pa.*

Accorded an A. Cressy Morrison Award in Natural Science
in 1957 by The New York Academy of Sciences



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Cumulative evidence from many sources has clearly established the thesis that the central nervous system exerts some regulation over anterior pituitary function. Harris¹⁻³ has marshaled the evidence from various experimental approaches (pituitary stalk section, transplantation, hypothalamic destruction, and excitation) and the hypothesis^{3,4} has been advanced that external stimuli modify the functional state of the adenohypophysis and its target glands by means of humoral agents elaborated by the hypothalamus. The hypophysial portal vessels are believed to constitute the anatomic link through which these as yet unidentified neurohumors gain access to the *pars distalis*. The concept that hypothalamus, hypophysis, and thyroid comprise an integrated neuro-endocrine mechanism often has been stated.^{5,6} It receives considerable support from recent experiments that provide evidence of a more direct nature than previously given for hypothalamic control of thyroid-stimulating hormone (TSH) secretion. Several groups of investigators⁷⁻¹³ have shown that electrolytic lesions in the anterior hypothalamus of the rat selectively interfere with TSH secretion by the adenohypophysis, with consequent reduction in some processes of thyroid function. Similar results have been obtained in the dog.¹⁴ It is significant also that the hypothalamic areas involved may be anatomically and functionally distinct; disturbance in one trophic mechanism can be produced with or without involvement of another.^{9-11, 15, 16}

Many facets of the hypothalamic-pituitary-thyroid interaction are by no means clear. It is the classic view that thyroid and adenohypophysis themselves constitute a "feedback" or servomechanism in which systemic stimuli (hormonal blood levels) govern the glandular interplay.¹⁷ The manner in which this basic interaction is integrated with the hypothalamus has defied analysis. The selective concentration of labeled thyroid hormones in the neurohypophysis¹⁸ suggests a direct action

*The work reported in this paper was supported by Grant A-432 from the National Institute of Arthritis and Metabolic Diseases, Public Health Service, Bethesda, Md.

†The junior author participated in the project on scholarships (Oncology Fund, Jefferson Medical College) during his third and fourth years as a medical student.

of circulating thyroid hormone on neural mechanisms, conceivably those regulating TSH secretion, although numerous species differences exist,^{18,19} and there is the probability that the concentration of thyroxine in the neurohypophysis is more concerned with mineral and water metabolism. Some have concluded that thyroid hormone regulates TSH release by direct action at the pituitary level.^{20,21} The experimental data are too scanty to permit acceptance of either view with confidence. The present study represents an experimental attempt to dissociate the neural and systemic components of the rat's hypothalamic-hypophysial-thyroid system so that the mechanisms can be analyzed individually. Specific answers to the following questions will be sought: Which phase of TSH secretion (production or release) is hypothalamically controlled? How much autonomous TSH function exists in the adenohypophysis after hypothalamic destruction? To what extent is thyroid hormone secretion affected in hypothalamic deficit? Is the thyroid-pituitary servomechanism under neural domination? Do blood levels of thyroid hormone regulate TSH secretion by direct action on the pituitary or via the hypothalamus? The basic approach will involve electrocoagulation of discrete hypothalamic areas and determination of change in various parameters of anterior pituitary-thyroid function, utilizing histochemical, bioassay, and radiometric techniques. Certain portions of the data have been published in preliminary form.^{12,22}

Materials and Methods

The bilaterally symmetrical electrolytic lesions were produced in the hypothalamus of adult female albino rats (Wistar strain; 200 to 350 gm. body weight) under ether anesthesia with a Krieg-Johnson stereotaxic apparatus. The over-all operative mortality was about 35 per cent. Approximately 100 animals survived brain electrocautery. The electrode, insulated except at its tip, was inserted through burr holes on the dorsum of the skull. Occasional bleeding from the sagittal sinus was controlled easily by compression. The lesion was placed 6 mm. anterior to the external auditory meatus, 0.5 mm. on either side of the midline, and 0.5 to 1 mm. from the base of the skull. The indifferent electrode was inserted in the rectum. A direct current of 4 ma. was applied for 15 to 30 seconds. A recovery period of at least 10 days was allowed before lesioned animals were utilized in further experiments. All rats received tap water and a ration of Purina Dog Checkers ad libitum. The placement of the lesions was subsequently checked by microscopic examination of Weil- and toluidine blue-stained sections (12 μ) made through the coronal plane of the diencephalic area of brains fixed in 10 per cent formalin for 5 days. The neuroanatomic descriptions of the rat's hypo-

thalamus by Gurdjian²³ and Krieg²⁴ were used for basic reference, and their terminology is retained for this study.

Thyroid secretion rate was assessed in 55 lesioned rats and 56 controls by an *in vivo* detection method²⁵ in which the biological decay of I^{131} from the gland was measured over the neck of the animal by a gamma-sensitive Geiger-Muller tube. Each rat received a single intraperitoneal injection of 20 to 25 μ c. of radioiodine 44 to 48 hours prior to the initial count. The initial count over the thyroid region, after appropriate correction for neck tissues, was considered to represent 100 per cent accumulation of radioactivity in the gland. Immediately following the initial count, each rat was injected with 10 mg. of propylthiouracil (PTU) to prevent subsequent recirculation of radioiodine through the thyroid. Counts were made at 24, 48, 72, and 96 hours thereafter. The per cent of radioactivity remaining in the gland (adjusted for isotopic decay) was plotted semilogarithmically against time to obtain a curve describing radioiodine release rate by the thyroid.

The servomechanism between adenohipophysis and thyroid was tested in 47 lesioned rats and 62 controls by determination of blood and pituitary TSH content after thyroid hormone administration. Thyroidal I^{131} release rates were obtained prior to hormone treatment in order to be certain that hypothalamic lesioning had definitely disturbed the pituitary-thyroid system. Lesioned rats, so screened, were then treated with varying dose levels of sodium L-triiodothyronine and sodium L-thyroxine in 0.9 per cent NaCl at pH 8.5. Animals in groups C, D, and E received, respectively, daily subcutaneous injections (0.5 ml.) of 0.4, 2, and 20 μ g. of triiodothyronine throughout the last 2 weeks of the postoperative period (24 to 31 days). Unoperated rats (series F, G, and H) were subjected to similar hormone treatment. Lesioned rats of group B were given saline injections. In another series of experiments lesioned rats were studied postoperatively for a 7- to 10-week period. Of these, some (group K) were injected with 10 μ g. of thyroxine once daily over the last 14 days of the experiment. Others remained untreated (series J). Appropriate control groups (I and L) were run in parallel experiments.

In order to test the capacity of the adenohipophysis to synthesize thyrotrophin in the face of hypothalamic damage, TSH concentration was measured in pituitaries that had been depleted of TSH prior to electrocautery. Advantage was taken of the fact that, in rats treated with propylthiouracil (PTU), a massive discharge of TSH from the pituitary occurs.¹⁷ Accordingly, rats were fed PTU in the diet (0.1 per cent) for 30 days. These goitrous rats were then lesioned and returned to the normal diet. PTU was similarly withdrawn from intact rats. Following goitrogen withdrawal (11 to 21 days), 32 lesioned and 29 intact rats were sacrificed and their hypophyses and blood were prepared for TSH assay.

At autopsy, blood was withdrawn from all rats by cardiac puncture under light ether anesthesia. Sera obtained from the pooled bloods of rats in each group were frozen until time of TSH assay. The hypophyses were quickly removed, the posterior lobes separated by blunt dissection, and the anterior lobes weighed to the nearest 0.1 mg. on a microtorsion balance. Pituitary extracts were made by homogenization and extraction of 7 to 10 pooled anterior lobes in acidified saline (0.25 per cent glacial acetic). Sera and pituitary extracts were bioassayed for TSH by a modification of the stasis tadpole method.^{26,27} Test tadpoles (*Rana clamitans*) were given injections of serum (0.2 ml.) or the equivalent of 0.2 to 0.3 mg. of anterior pituitary over a 4-day injection period. The response of the tadpole thyroid was measured microhistometrically as increase in acinar cell height. TSH content in the sample tested was calculated by extrapolation from a standard TSH dose-response curve (FIGURE 1). The standard curve was obtained by serial dilution of the U.S.P. TSH Standard in acidified saline. The data on which the standard dose-response curve is based were subjected to an analysis of variance in conformance with accepted statistical procedures.^{28,29} One hundred and twenty-six

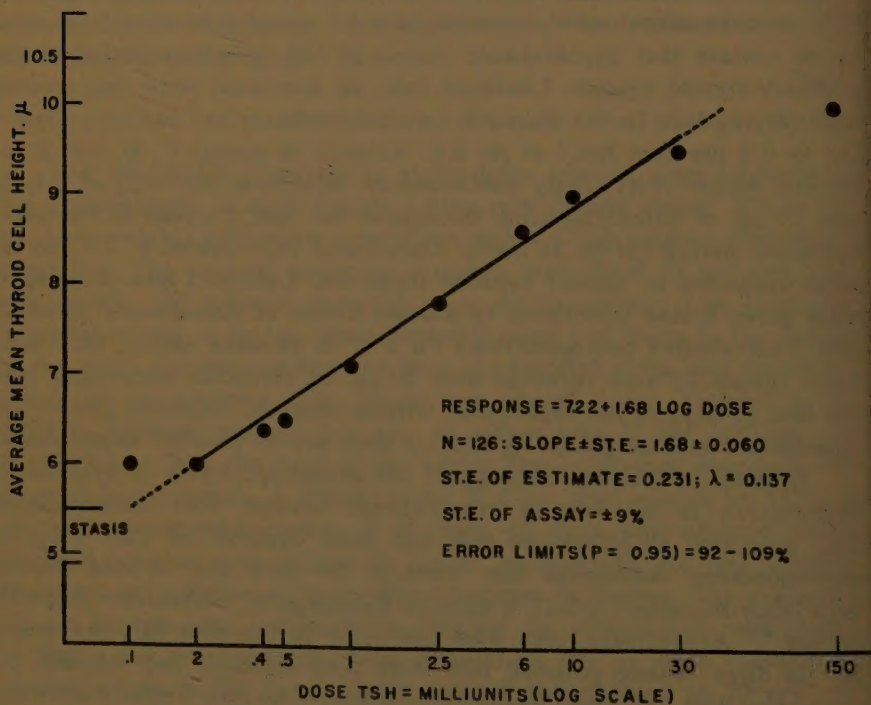


FIGURE 1. Bioassay of U.S.P. TSH Standard in the stasis tadpole.

tadpoles and 10 different dose levels of the standard, ranging from 0.1 to 150 milliunits, were used. The equation for the line, estimated by the method of least squares, was $Y = 7.22 \pm 1.68 \log \text{dose}$. The standard deviation about the line (standard error of estimate) was 0.231, with an index of precision (λ) equal to 0.137. The standard error of the assay was found to be ± 9 per cent, with fiducial limits ($P = 95$) of 109 to 92 per cent. Routinely, 7 to 9 tadpole thyroids were measured in each bioassay group. With this number of test animals, the method successfully differentiated (95 per cent level of confidence) 2 doses of TSH in a ratio of 1.35 to 1.0. A comparison of these data with those derived from other bioassay procedures on pituitary trophic hormones^{28,30} reveals the high order of sensitivity and precision afforded by the stasis tadpole technique.

At sacrifice, whole pituitaries, thyroids, adrenals, and ovaries were weighed, fixed, and then prepared for histological study by paraffin embedding. The cellular structure of the adenohypophysis was evaluated on Helly-fixed, serial sections cut at 3 and 4 μ through the horizontal plane. Masson staining (acid fuchsin, ponceau de xylydine, aniline blue) was used for routine differentiation of the cellular types in the anterior pituitary. Further identification of the basophil varieties ("beta" or "thyrotrophs," "delta" or "gonadotrophs")* was accomplished by staining with the periodic acid-Schiff (PAS) technique for glycoproteins (Purves and Griesbach³¹) and with the aldehyde-fuchsin stain of Gomori.³²

Results

A systematic study of the fiber and nuclear stained sections through the diencephalon of lesioned rats disclosed extensive damage to the hypothalamus (FIGURES 7 and 8). The heart of the lesion consisted of a necrotic mass, 1 to 2 mm. in diameter, representing confluence in the midline of both areas of electrolysis. Typically, the lesion was

*The thyrotrophs^{31,35,36} (beta cells)^{33,34} believed to elaborate TSH are situated in the central portion of the anterior lobe, are angular in shape, and stain readily with both PAS and aldehyde-fuchsin (AF) methods. In thyroid hormone deficiency, these beta cells lose most of their glycoprotein granulation, are rendered PAS and AF negative, and transform into multivacuolated hypertrophic basophils called "thyroidectomy" cells. Contrariwise, the gonadotrophs (delta cells) are thought to produce follicle-stimulating hormone and luteinizing hormone. Their distribution in the gland is mainly peripheral, with concentrations in a dorsal zone adjacent to the *pars intermedia* and in a wide zone along the inferior aspect of the anterior lobe. These cells are round or oval in shape, usually bear prominent negative images, and are PAS reactive, but stain poorly with aldehyde fuchsin. After gonadectomy, this cell type responds by marked increase in glycoprotein content (PAS positive) and by acquisition of a large hyaline vacuole. Eventually, the cell assumes the appearance of a signet ring and is then referred to as a "castration" cell. In properly stained sections with the Masson technique, both thyrotrophs and gonadotrophs are easily differentiated (FIGURES 2-5, PLATE I, between pages 254 and 255; FIGURE 6).

basally located in the anterior and tuberal portions of the hypothalamus. Its rostral extreme occasionally reached the paraventricular nucleus and, more infrequently, the suprachiasmatic (ovoideus) nucleus. The supraoptic nucleus was rarely, if ever, damaged. Caudally, the lesion extended into the posterior hypothalamus to enroach on the mammillary nuclear complex. Posterior hypothalamic damage was relatively slight, however. Ventricular (third) relationships were completely disrupted, and some destruction of neighboring fiber tracts (fornix, mamillothalamic, and medial forebrain bundle) always resulted. The most variable aspect of lesion placement was its depth. In some instances there was destruction of the floor of the hypothalamus with complete disorganization of the median eminence. More frequently, impingement on the median eminence was slight. The anterior hypothalamic, dorsomedial, and ventromedial nuclei invariably suffered injury from the electrocautery, the arcuate nucleus inconsistently so.

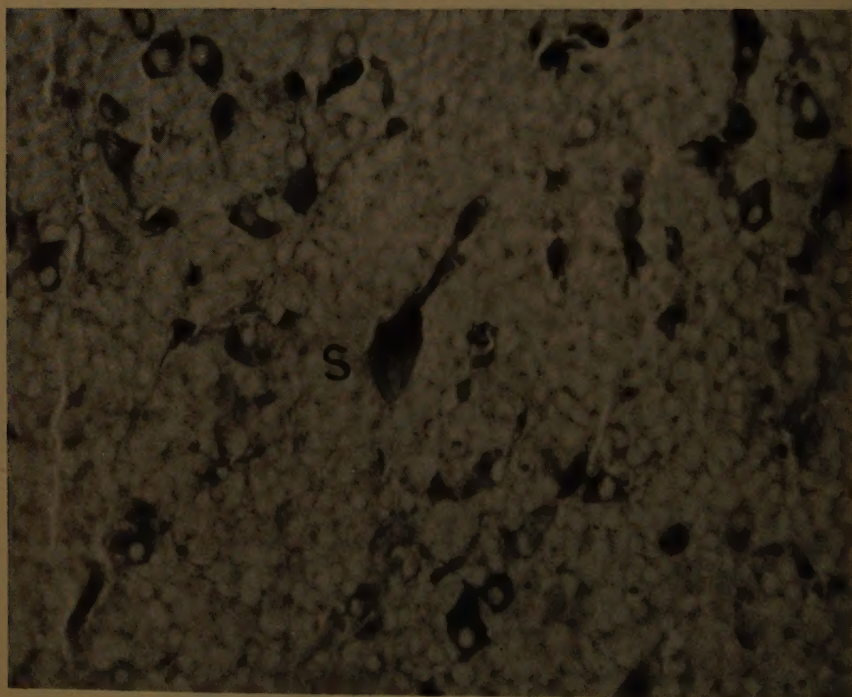


FIGURE 6. The selective affinity of the beta cells for aldehyde fuchsin, demonstrated by intense staining of glycoprotein cytoplasmic granulation. These AF-positive cells are usually at some distance from the large sinusoids (S). Gomori, aldehyde fuchsin. $\times 400$.

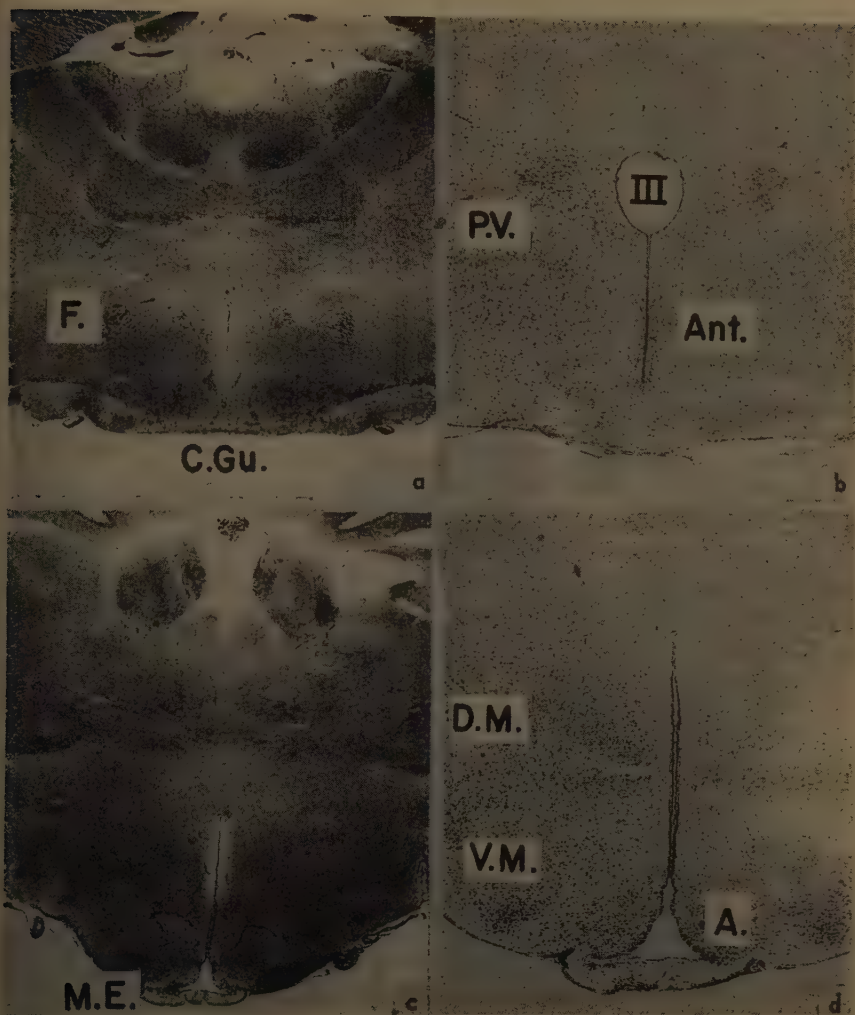


FIGURE 7. Coronal sections through the diencephalic area of the rat's brain, showing the normal anatomy of the hypothalamus for those regions usually affected by electrocautery. (a) Section through the anterior third of the diencephalon at the level of the paraventricular nucleus (Weil, $\times 12$); (b) typical winglike configuration of the paraventricular nucleus as revealed by toluidine blue staining ($\times 24$); (c) section through the rostral portion of the middle third of the hypothalamus (Weil, $\times 12$); (d) area of the tuberal portion of the hypothalamus at the level of maximal development of the median eminence—dorso-medial, ventromedial (posterior portion), and arcuate nuclei are indicated (toluidine blue; $\times 24$).

A statistical treatment of the results (TABLE 1; FIGURE 9) indicates that brain electrocautery produced a significant decrease in relative and absolute weight of the anterior pituitary. The adenohypophyses of rats bearing extensive lesions in the anterior and tuberal hypothalamus were consistently smaller than those of intact controls (compare groups

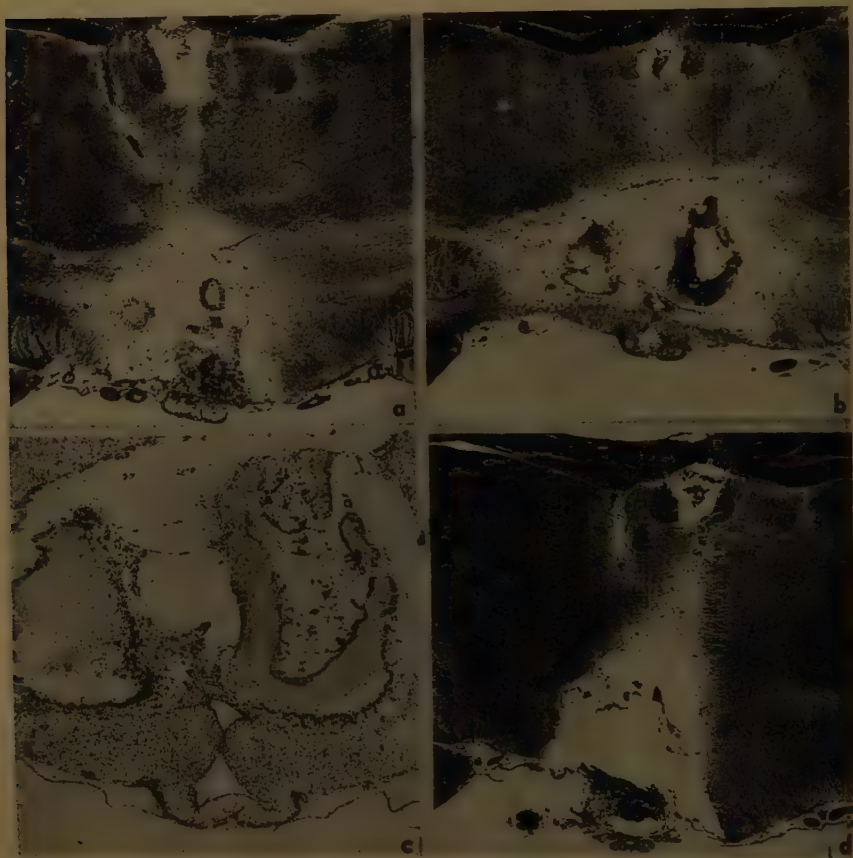


FIGURE 8. Types of lesions produced in this study (the rostral and caudal limits of the lesions depicted are indicated in TABLE 2). (a) Hypothalamic lesion of an obese rat (Ji, TABLE 2) in which thyroid and ovary were affected. The lesion impinges on the median eminence, but the pituitary stalk and the surrounding blood vessels are intact (Weil, $\times 12$); (b) hypothalamic lesion in a rat (Jo, TABLE 2) that failed to become obese and in which thyroid function was affected (Weil, $\times 12$); (c) morphologic integrity of the arcuate nucleus and median eminence in the lesion shown in 7b (toluidine blue, $\times 24$); (d) destructive lesion that produced obesity and panhypopituitarism in rat Jr (TABLE 2). The area of necrosis has broken through the floor of the hypothalamus and extends dorsally into the thalamus (Weil, $\times 12$).

TABLE 1

EFFECT OF EXOGENOUS THYROID HORMONE ON THE
PITUITARY-THYROID SYSTEM OF HYPOTHALMIC LESIONED RATS

Group	No. of animals	Mean body weight (gm.)			Days lesioned	Mean gland weight (mg.)		TSH assay of adenohypophysis*	
		At operation	Prior to treatment	At sacrifice		Thyroid	Adenohypophysis	Cell height (μ)	TSH per mg.
A Normal untreated	30			247 $\pm$ 38†	0	15.6 $\pm$ 0.5	11.0 $\pm$ 0.4	9.4 $\pm$ 0.27	66 (40-100)‡
B Lesioned + saline	14	236 $\pm$ 9	215 $\pm$ 29	228 $\pm$ 36	24-26	14.4 $\pm$ 0.7	8.4 $\pm$ 0.9	9.2 $\pm$ 0.24	80 (48-120)
C Lesioned + 0.4 μ g. T ₃ §	10	236 $\pm$ 8	296 $\pm$ 24	334 $\pm$ 38	27-31	17.4 $\pm$ 1.0	9.5 $\pm$ 0.6	8.2 $\pm$ 0.21	20 (15-28)
D Lesioned + 2 μ g. T ₃	13	313 $\pm$ 19	270 $\pm$ 34	273 $\pm$ 27	24-32	17.4 $\pm$ 1.3	11.5 $\pm$ 1.3	6.4 $\pm$ 0.10	1.8 (1.5-2.0)
E Lesioned + 20 μ g. T ₃	11	311 $\pm$ 35	288 $\pm$ 34	249 $\pm$ 36	24-31	17.7 $\pm$ 1.0	9.9 $\pm$ 0.6	5.4 $\pm$ 0.09	0
F Normal + 0.4 μ g. T ₃	14		290 $\pm$ 23	287 $\pm$ 22	0	17.4 $\pm$ 0.9	12.9 $\pm$ 0.9	8.0 $\pm$ 0.25	15 (13-22)
G Normal + 2 μ g. T ₃	18		234 $\pm$ 21	249 $\pm$ 18	0	14.8 $\pm$ 0.8	10.9 $\pm$ 0.8	5.7 $\pm$ 0.13	0.6 (0-0.8)
H Normal + 20 μ g. T ₃	15		242 $\pm$ 31	238 $\pm$ 22	0	13.7 $\pm$ 0.7	10.5 $\pm$ 0.8	5.8 $\pm$ 0.16	0.8 (0.5-0.95)
I Normal untreated	12			290 $\pm$ 40	0	17.8 $\pm$ 1.4	12.5 $\pm$ 0.9	9.1 $\pm$ 0.16	70 (60-90)
J Lesioned untreated	12	266 $\pm$ 12		376 $\pm$ 60	60-67	17.3 $\pm$ 0.6	9.7 $\pm$ 0.9	9.0 $\pm$ 0.17	63 (45-80)
K Lesioned + 10 μ g. T ₄ §	17	242 $\pm$ 10	296 $\pm$ 54	306 $\pm$ 50	48-64	15.4 $\pm$ 0.7	8.1 $\pm$ 0.7	6.4 $\pm$ 0.15	1.8 (1.4-2.0)
L Normal + 10 μ g. T ₄	15		308 $\pm$ 25	305 $\pm$ 23	0	18.9 $\pm$ 1.2	12.9 $\pm$ 1.0	6.2 $\pm$ 0.12	1.3 (1.0-1.5)

*Test tadpoles received the equivalent of 0.2 to 0.3 mg. of pituitary over a 4-day injection period.

†Mean body weight $\pm$ average deviation; in all other columns the $\pm$ values represent the standard error of the mean.

‡Mean TSH concentration in U.S.P. millunits; in parentheses, lower and upper limits of detection are calculated from standard error of each assay.

§T₃ = triiodothyronine; T₄ = thyroxine.

A and B, I and J, and K and L). The diminution in hypophyseal weight was more pronounced in rats lesioned for longer periods of time and for those in which hyperphagia and obesity occurred (series C and J). In some rats hyperphagia was noted within several days after the operation. Such lesioned animals almost doubled their body weight during the post-

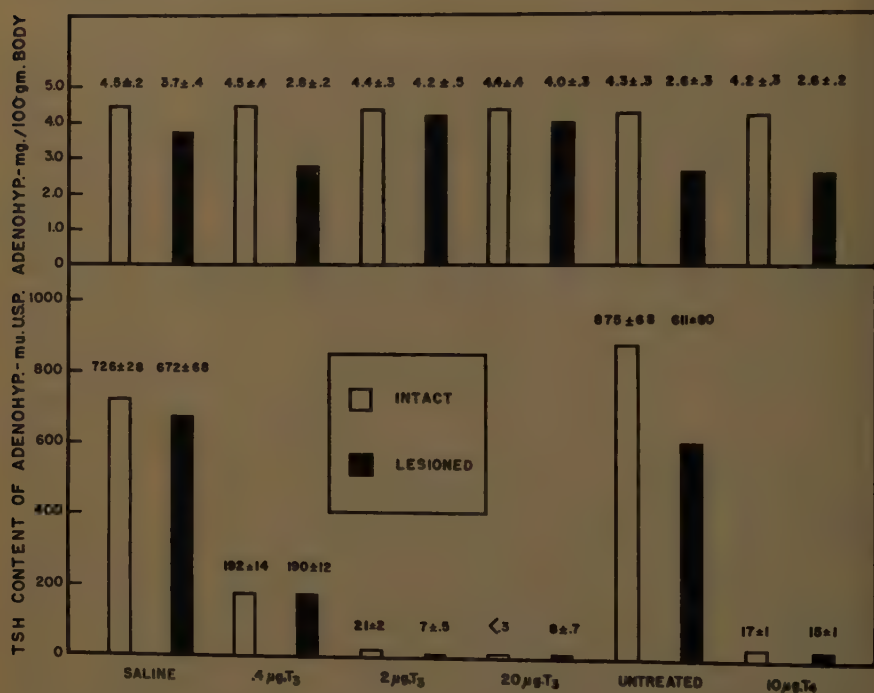


FIGURE 9. TSH content of the adenohypophysis after thyroid hormone treatment. The upper graph compares adenohypophyseal-body weight ratios $\pm$ standard errors of the mean in intact and lesioned rats with and without thyroid hormone treatment (based on data in TABLE 1). In the lower graph the mean TSH content (mean weight $\times$ mean concentration) of the adenohypophysis, expressed in U.S.P. milliunits $\pm$ standard error of the mean, is correspondingly illustrated.

operative period (TABLE 2). Hypophyseal weight change in obese rats became more apparent when comparison was made with "weight" rather than "age" controls (TABLE 3). Mean adenohypophyseal weight in animals of group X (old normal rats) was 15.2 ± 0.8 mg. Calculation of adenohypophyseal-body weight ratios revealed a decrease in obese lesioned rats (group J) approximating 35 per cent. The weight decrement with whole pituitaries was still greater inasmuch as posterior lobe atrophy also occurred in the hypothalamic lesioned animal. Although

TABLE 2
LESION ANALYSIS AND ENDOCRINE RESPONSE

Rat	Body weight (gm.)		Morphologic change (wt.)			Thyroid ¹³¹ I Release	Hypothalamic lesion*
	Pre-operative	Final	Adeno- hypophysis	Adrenal	Ovary		
Jm	274	448	Atrophy	Normal	Atrophy	Delayed	From PV to IPF; ME destroyed. From SC to MB through floor of V hypothalamus. From PV to PreMB; ME destroyed but adj. blood vessels intact. Basal, asymmetrical, VM to MB; ME intact. PV to PreMB; ME intact. Asymmetrical, PV to MB; ME intact. Asymmetrical, VM to MB; ME intact. VM to MB; ME intact. VM to MB; ME destroyed. VM to MB; ME partial destruction. PV to PreMB; ME intact.
Jr	290	484	Atrophy	Atrophy	Atrophy	Delayed	
Jg	302	416	Atrophy	Normal	Atrophy	Delayed	
Je	272	445	Atrophy	Normal	Atrophy	Delayed	
Ji	276	510	Atrophy	Normal	Atrophy	Delayed	
Jk	270	446	Normal	Normal	Normal	Delayed	
Jn	262	240	Normal	Normal	Hypertrophy	Increased	
Jo	262	300	Atrophy	Normal	Normal	Delayed	
Jq	258	385	Atrophy	Normal	Atrophy	Delayed	
Jp	265	350	Atrophy	Atrophy	Atrophy	Normal	
Jl	256	320	Normal	Atrophy	Atrophy	Delayed	

*Brain abbreviations: SC, suprachiasmatic nucleus; PV, paraventricular nucleus; VM, ventromedial nucleus; DM, dorsomedial nucleus; A, arcuate nucleus; Ant, anterior hypothalamic nucleus; MB, mammillary body; PreMB, pre-mammillary nuclei; ME, median eminence; IPF, interpeduncular fossa; CGu, commissure of Gudden; F, fornix; M, mammillothalamic tract; III, third ventricle.

TABLE 3
DIFFERENTIAL RESPONSE OF ADRENAL AND OVARY TO
EXOGENOUS THYROID HORMONE IN HYPOTHALAMIC LESIONED RATS

Group*	Treatment	Mean body weight (gm.)	Mean organ weight (mg.) 100 gm. body weight			
			Adrenal	Level of significance†	Ovary	Level of significance†
Experiment 1 Normal (I) Lesioned (J) § Lesioned (K) Normal (L) Normal (X)	None	290 ± 40 ‡	20.9 ± 1.1	P < 0.02 P n.s. P < 0.005	25.8 ± 2.3	P < 0.005 P n.s. P < 0.001
	None	376 ± 60	16.4 ± 1.3		16.5 ± 1.3	
	10 µg. T ₄	306 ± 50	18.2 ± 0.8		16.9 ± 1.5	
	10 µg. T ₄	305 ± 23	22.9 ± 1.0		30.7 ± 1.6	
	None	386 ± 32	15.4 ± 0.9		26.8 ± 2.4	
Experiment 2 Normal (A) Lesioned (B) Lesioned (E) Normal (H) Lesioned (D) Normal (G) Lesioned (C) § Normal (F)	None	247 ± 38	22.5 ± 0.7	P n.s. P n.s. P n.s. P n.s. P < 0.001	25.9 ± 1.1	P < 0.005 P < 0.001 P < 0.001 P < 0.001 P < 0.001
	Saline	228 ± 36	21.0 ± 1.1		20.4 ± 1.4	
	20 µg. T ₃	249 ± 36	36.7 ± 2.6		23.5 ± 2.8	
	20 µg. T ₃	238 ± 22	33.0 ± 1.2		38.8 ± 1.7	
	2 µg. T ₃	273 ± 27	27.6 ± 1.4		23.4 ± 1.7	
	2 µg. T ₃	249 ± 18	26.7 ± 0.9		36.5 ± 1.5	
	0.4 µg. T ₃	334 ± 38	17.2 ± 0.9		19.8 ± 1.4	
	0.4 µg. T ₃	287 ± 22	22.9 ± 0.7		27.0 ± 1.3	

*The lettered groups correspond to those in TABLE 1; group X represents a normal untreated "weight" control group to group J (obese).

† Statistical significance calculated from *t* test tables; P values > 0.05 are not deemed significant (n.s.)

‡ Body weight ± average deviation at sacrifice; in other columns the ± values refer to standard error of the mean.

§ Animals in these groups were obese.

absolute thyroid weight was not appreciably reduced by electrocautery, thyroid-body weight ratios were substantially decreased in lesioned rats that became obese. Moreover, evaluation of the gland's histology, as well as measurement of thyroidal I^{131} release rate, disclosed significant diminution in thyroid activity. Acinar cell height was decreased, and there was evidence of colloid retention (FIGURES 10 and 11). These

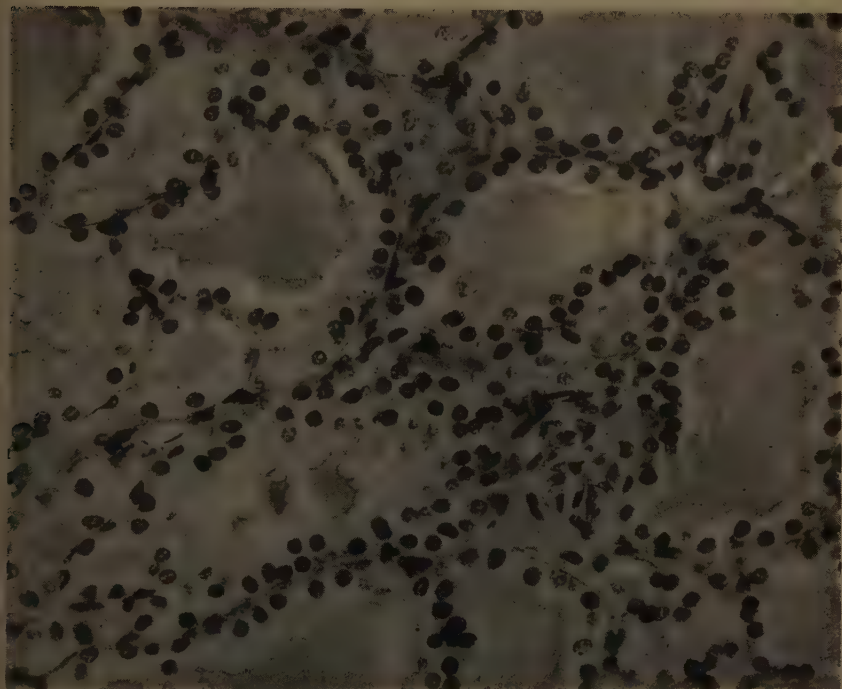


FIGURE 10. Section in the central area of the thyroid, showing the typically tall columnar follicular epithelium and liquefied nature of the colloid in the normal gland. Harris's hematoxylin and eosin. $\times 400$.

morphologic alterations were correlated readily with changes in thyroidal radioiodine release. Mean percentages of radioactivity remaining in the thyroids of lesioned rats were significantly greater than in controls at all time intervals measured (FIGURE 12). The delay in I^{131} discharge by the thyroid was apparent as early as 12 days after lesioning and was maintained thereafter. Release curves were essentially similar in rats lesioned 30 to 50 days prior to measurement. Biological half lives for control and lesioned animals were 46 and 86 hours, respectively. The delay in thyroidal radioiodine discharge was greater in those lesioned rats that became obese.

Despite reduction in weight of the anterior pituitary, the mean TSH concentration in the smaller gland of lesioned rats remained within normal limits. No significant alteration in TSH concentration was noted in animals differing only as to duration of the lesion (series B and J); in each instance, TSH levels were essentially similar to that of the appropriate control group. Nevertheless, it was clear that hypothalamic destruction did result in significant change in the dynamics of adeno-



FIGURE 11. Histological evidences of reduced thyroid secretion in the gland of a lesioned rat (55 days postoperatively). There is decreased vacuolation of colloid and moderate reduction in acinar cell height. Harris's hematoxylin and eosin stain. $\times 400$.

hypophysial TSH secretion. Mathematical conversion of TSH concentrations in the gland to total hormone content (mean TSH concentration $\times$ mean gland weight) indicated a reduction in adenohypophysial stores of the lesioned rat from a normal value averaging 800 U.S.P. milliunits per gland to approximately 650 milliunits. TSH blood levels also were decreased significantly in all lesioned groups. The simultaneous presence of decreased levels of circulating thyrotrophin and reduced hormonal stores in the pituitary indicated that both production and release phases

PLATE I

FIGURE 2. Area in the dorsal portion of the normal gland, in which pale blue oval basophils (delta cells) are clustered around the larger sinusoids. The light halos in the cytoplasm represent the negative Golgi images. Masson. $\times 400$.

FIGURE 3. Transformation of the delta basophils to "castration" cells after gonadectomy (1 year) recorded in the dorsal zone adjacent to the *pars intermedia* (visible at the lower right). Masson. $\times 200$.

FIGURE 4. Intermingling of deeply stained angular basophils (beta cells) and the pale oval ones (delta cells) in the ventral region of the *pars distalis*. Masson. $\times 400$.

FIGURE 5. An area in the anteromedian portion rich in angular basophils (beta). Compare with FIGURE 6, representing an adjacent section. Masson. $\times 400$.

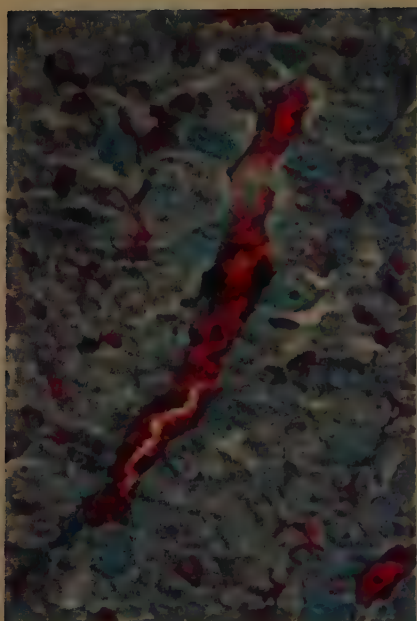


FIGURE 2

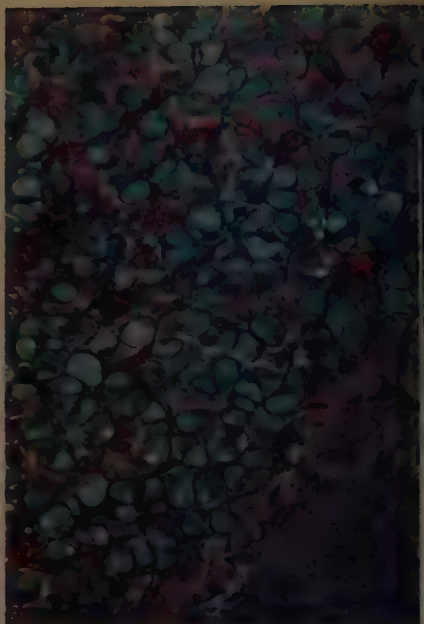


FIGURE 3



FIGURE 4

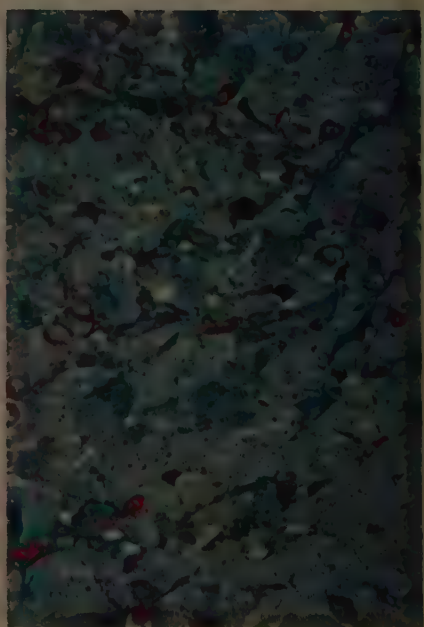


FIGURE 5

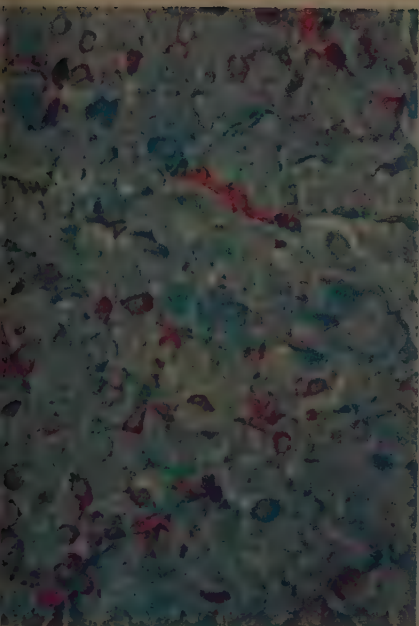


FIGURE 14

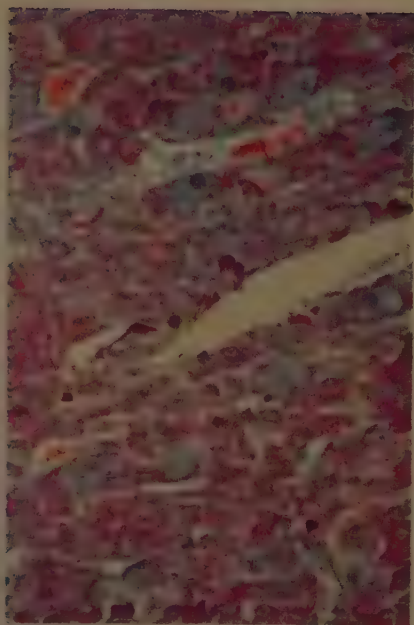


FIGURE 16



FIGURE 17

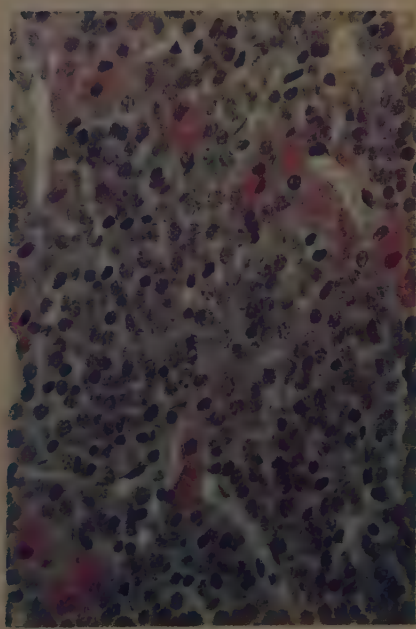


FIGURE 18

PLATE II

FIGURE 14. Cellular condensation in the adenohypophysis of the lesioned rat (Jo, TABLE 2). There are regressive changes in the acidophils and basophils. Beta and delta basophils are still present, however. Masson. $\times 400$.

FIGURE 16. Effect of triiodothyronine administration on the cellular elements of the adenohypophysis of a lesioned rat. Regranulation of acidophils has occurred. There is a striking increase in the numbers of pale blue oval basophils (delta cells) near the large vascular channels. Masson. $\times 400$.

FIGURE 17. Marked increase in numbers of basophils after thyroxine administration to lesioned rats. Virtually all of the basophils are of the delta category; few, if any, angular basophils are observed. Masson. $\times 400$.

FIGURE 18. Histochemical reactivity of the basophils in FIGURE 17 (nearby area). The oval cells with prominent negative images stain intensely (reddish pink) for glycoprotein with the periodic acid-Schiff technique. They are aldehyde-fuchsin negative. PAS; Harris's hematoxylin counterstain. $\times 400$.

of thyrotrophic hormone secretion were likewise affected in rats bearing hypothalamic lesions. However, the still ample reserves of TSH in the pituitary and the moderate degree of change in the thyroid signified a high measure of residual TSH function in the "isolated" pituitary.

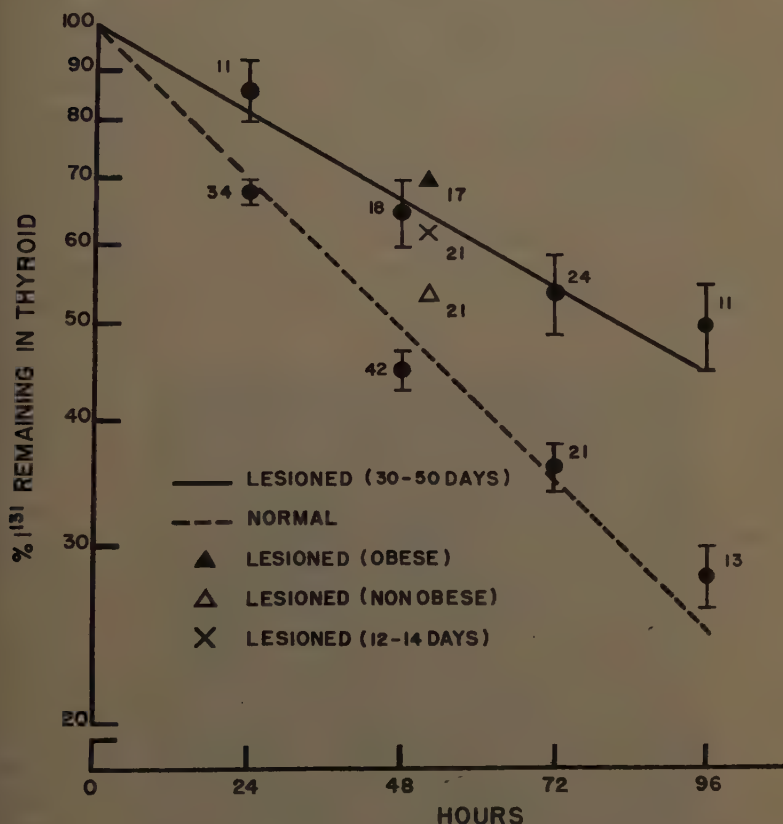


FIGURE 12. Semilogarithmic plot of the mean per cent of I^{131} remaining in the thyroid in lesioned rats after "0" time (time of initial count, 44 to 48 hours after I^{131} injection, at which thyroidal I^{131} accumulation is considered to be 100 per cent). The vertical bars depict 1 standard error on either side of the mean. The number of determinations (1 per animal) establishing each point is also indicated.

As expected, the administration of triiodothyronine and thyroxine to intact rats profoundly affected the pituitary-thyroid system (TABLE 1; FIGURE 9). Both hormonal agents were found to inhibit TSH secretion by the pituitary. Although the relative effectiveness of these thyroid hormones was not thoroughly tested, it was evident that, on a weight

basis, triiodothyronine was much more potent than thyroxine in suppression of TSH secretion. The lowest dose of triiodothyronine used ($0.4\text{ }\mu\text{g.}$) reduced TSH content of the pituitary to approximately 25 per cent of normal; with higher dose levels the depletion was virtually complete. The $10\text{-}\mu\text{g.}$ and $2\text{-}\mu\text{g.}$ dose levels of thyroxine and triiodothyronine, respectively, were about equipotent. Thyroid hormone administration to lesioned rats reduced thyrotrophin stores in the pituitary as drastically as in controls. The response of the pituitary to exogenous thyroid was qualitatively and quantitatively similar to that in the intact animal. Adenohypophysial TSH content in lesioned rats was decreased 97 to 99 per cent with either thyroxine ($10\text{-}\mu\text{g.}$ dose level) or triiodothyronine ($2\text{ }\mu\text{g.}$). The thyroid glands of rats receiving exogenous thyroid hormone invariably approached the resting condition (FIGURE 13). Blood TSH titers, furthermore, were correspondingly decreased nearly to hypophysectomy levels in both lesioned and intact rats. The results thus clearly

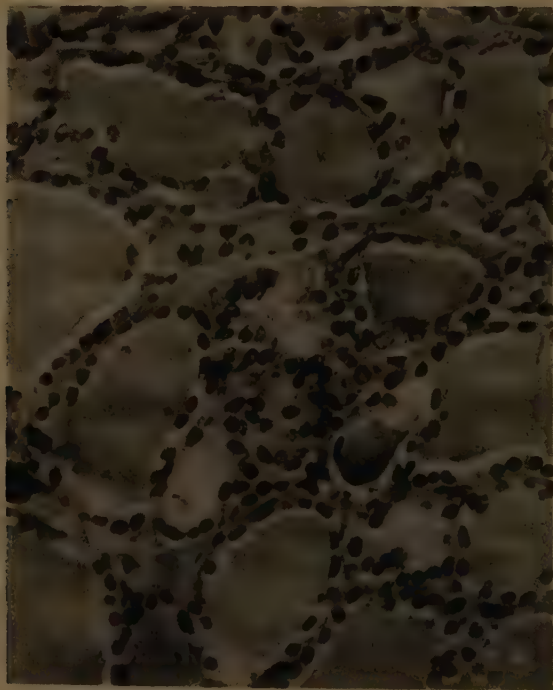


FIGURE 13. Effect of triiodothyronine administration on the structure of the thyroid in a lesioned rat (30 days postoperatively). Histological regression is marked; follicles are distended with dense, homogeneous colloid, and the acinar epithelium is reduced to the cuboidal or squamous state. Harris's hematoxylin and eosin. $\times 400$.

indicated that high blood levels of thyroid hormone can act directly on the hypophysis, and that the servomechanism between the thyroid and anterior pituitary was able to function in the presence of considerable hypothalamic damage.

The experimental results with propylthiouracil are shown in TABLE 4. In rats made goitrous with PTU, subsequent lesioning did not prevent reaccumulation of TSH by the adenohypophysis when the goitrogen was withdrawn. In fact, TSH concentrations in the pituitary rebounded to supernormal levels before the normal condition was eventually achieved (20 days). This rebound phenomenon was much the same in lesioned and intact rats. The course of blood TSH levels after goitrogen withdrawal differed significantly, however. TSH serum titers in intact rats receded from a peak concentration to normal levels, which were maintained thereafter. On the other hand, TSH serum titers in lesioned rats could not be maintained, and they eventually fell to subnormal levels. The rate at which involution occurred in the hyperplastic thyroids after goitrogen withdrawal was much the same, however.

Atrophy of the *pars nervosa* was observed frequently in lesioned rats. The shrunken fibrous mass contained much hyaline amorphous material that stained readily with PAS or aldehyde-fuchsin techniques. Hypertrophic changes in the *pars intermedia* usually accompanied the atrophic changes in the *nervosa*. There was cellular enlargement, with activation of nuclei. Glycoprotein staining was highly intensified.

Aside from the occasional presence of relatively small circumscribed areas of necrotic cells, the histological appearance of the adenohypophysis in lesioned rats was generally healthy (FIGURES 2-5, PLATE I). The occurrence of pituitary infarcts was infrequent and their size surprisingly small, even with lesions that destroyed the floor of the hypothalamus. The vascular anatomy was characterized by marked sinusoidal distension, with some evidence of venous stasis. Considerable shrinkage of all cell types in the parenchyma contributed to the diminished size of the anterior pituitary. Both cytoplasm and nucleus were affected (FIGURE 14, PLATE II). The reduction in volume of the chromophobes and acidophils and the degranulation of the latter were more conspicuous than in basophils. It is significant that even with the most destructive lesion the angular basophils, staining deeply with aniline blue and showing selective affinity for aldehyde fuchsin, persisted in the anterior lobe (FIGURE 15). The delta basophils and the acidophils responded more sensitively to hypothalamic lesioning. The alterations in these cell types could be correlated with placement of the lesion and functional activity of the target endocrines. With lesions that disorganized the floor of the hypothalamus and completely destroyed the median eminence, the regression in the acidophils was profound (FIGURE 15). In lesioned

TABLE 4
PITUITARY-THYROID PARAMETERS IN GOITROUS RATS AFTER
HYPOTHALAMIC LESIONING AND PROPYLTHIOURACIL WITHDRAWAL

Treatment	No. of rats	Body weight (gm.) at sacrifice	Organ weight (mg.)		TSH assay			
			Thyroid	100 gm. body weight	Serum cell height (μ)	TSH/ml.*	Adenohypophysis cell height (μ)	TSH/mg.*
On PTU, 30 days	11	271 $\pm$ 35 [†]	22.4 $\pm$ 2.5	4.5 $\pm$ 0.30	6.3 $\pm$ 0.10	1.6	6.6 $\pm$ 0.30	2.3
On PTU, 30 days Off PTU, 11 days	8	256 $\pm$ 49	11.9 $\pm$ 0.9	3.9 $\pm$ 0.33	5.7 $\pm$ 0.11	0.6	10.2 $\pm$ 0.28	250
On PTU, 30 days Lesioned Off PTU, 12 days	8	229 $\pm$ 22	10.7 $\pm$ 1.5	4.2 $\pm$ 0.70	5.9 $\pm$ 0.07	0.9	10.2 $\pm$ 0.39	250
On PTU, 30 days Off PTU, 20 days	10	293 $\pm$ 44	8.5 $\pm$ 0.4	4.9 $\pm$ 0.36	6.0 $\pm$ 0.10	1.0	9.3 $\pm$ 0.31	100
On PTU, 30 days Lesioned Off PTU, 21 days	10	250 $\pm$ 32	9.7 $\pm$ 0.5	3.9 $\pm$ 0.31	5.5 $\pm$ 0.10	<0.4	9.2 $\pm$ 0.30	80
No drug Lesioned, 24-26 days	14	228 $\pm$ 36	6.3 $\pm$ 0.3	3.7 $\pm$ 0.37	5.5 $\pm$ 0.09	<0.4	9.3 $\pm$ 0.24	100
Normal food Intact	12	290 $\pm$ 40	6.1 $\pm$ 0.5	4.3 $\pm$ 0.31	6.0 $\pm$ 0.06	1.0	9.1 $\pm$ 0.16	70

*Mean TSH concentration expressed in U.S.P. millunits.

[†]Body weight $\pm$ averaged deviation at sacrifice; in other columns the $\pm$ values refer to standard error of the mean.

[‡]Statistical significance calculated from *t* test tables. P values > 0.05 are not deemed significant (n.s.).

rats with median eminence intact and arcuate nucleus undamaged, the histological picture of the acidophils was reminiscent of that in mild thyroid hormone deficiency, namely, the presence of numerous sparsely granulated acidophilic cells (FIGURE 14, PLATE II). The gonadotrophic basophils (delta cells) displayed the greatest change in rats with ovarian atrophy. The characteristic clustering of this cell type along the portal vessels and larger sinusoids was absent. Only a few PAS-positive cells of this category could be found in the dorsal zone adjacent to the *pars intermedia*. Delta cells were present in ventral regions of the gland, although their glycoprotein granulation was definitely reduced. Thyroid hormone administration to lesioned rats induced striking alterations in the proportion and fine structure of the chromophilic cells in the adenohypophysis. Numerous acidophils in all stages of regranulation were found, and staining intensity in this cell type was increased (FIGURE 16, PLATE II). There was almost complete disappearance of thyrotrophs. The loss of the beta cells in the basophilic population was more than compensated by a substantial increase in the numbers of delta cells in both dorsal and ventral portions of the anterior lobe (FIGURES 16 and 17, PLATE II). These cells were identified readily as gonadotrophs by their affinity for PAS staining, their refractoriness to aldehyde fuchsin, their prominent negative images, and the intimate relationship to blood channels (FIGURE 18, PLATE II).

The effect of hypothalamic lesioning on adrenal and ovary and the differential response of these target endocrines to thyroid hormone treatment are shown in TABLE 3. Hypothalamic destruction did not affect the target endocrines with equal severity. Ovarian atrophy was usually marked in lesioned rats that became obese (compare series J with I and X). Adrenal atrophy, on the other hand, was the exception rather than the rule. Adrenal-body weight ratios in obese lesioned rats were lower than in intact rats of similar age, but comparison with heavier normal rats (group X) revealed no significant weight change. Absolute reduction in adrenal weight did occur in some rats, but analysis of lesion placement (TABLE 2; FIGURE 8) revealed no consistent difference in the character and extent of hypothalamic damage. More frequently, ovarian atrophy and reduced thyroid function were present simultaneously, particularly with lesions that were basally located so as to damage arcuate nucleus and median eminence (FIGURE 8). Dissociation of these effects on thyroid and ovary could be accomplished by placement of the lesion in the dorsal hypothalamus (electrode tip 2 mm. from the skull base). With this type of lesion, ovarian, adrenal, and pituitary weights remained normal, but the hyperplastic response of the thyroid to a goitrogen was partially inhibited (thyroidal I^{131} release was not tested here).

The differential response of adrenal and ovary to hypothalamic destruction became more apparent after administration of thyroid hormone (TABLE 3). The 2- and 20- μ g. dose levels of triiodothyronine produced substantial hypertrophy in both adrenal and ovary of intact animals. The increase in adrenal-body weight ratios was approximately 20 and 50 per cent with 2 and 20 μ g. of triiodothyronine, respectively. The ovarian enlargement was approximately the same (50 per cent) with both dose levels. Similar triiodothyronine treatment in lesioned rats resulted in an adrenal hypertrophy of equal magnitude, but failed to reverse the atrophic changes in the ovary. The ovarian response with thyroxine was much the same, although the 10- μ g. dose used was apparently insufficient to cause more than moderate change in adrenal size.

Discussion

Greer's demonstration^{7,8} that electrolytic lesions in the anterior hypothalamus of the rat inhibit the hyperplastic response of the thyroid to propylthiouracil without impairment of iodide-trapping function led him to postulate the existence of two thyrotrophins in the pituitary—one under hypothalamic control, regulating thyroid morphology ("growth factor"), the other, a "metabolic factor" independent of neural influence, enabling concentration and binding of iodine by the thyroid. This concept of TSH duality was supported by results from transplantation studies in which anterior pituitary grafts to eyes of hypophysectomized mice maintained normal I^{131} uptakes in atrophic thyroids.³⁷ Bogdanove and his associates^{10,11} confirmed and extended the findings of Greer, but rejected the principle of two thyrotrophins. It was emphasized that the dichotomy between morphologic and physiologic response of the thyroid after hypothalamic lesioning was better explained by the fact that propylthiouracil markedly potentiates the stimulating effect of TSH on the iodide pump without altering thyroid structure.³⁸ The results of the present study, while providing direct evidence for hypothalamic control of TSH secretion, make the existence of two thyrotrophins still less likely. The regressive histological changes in the thyroid and the delayed rate of discharge of radioiodine from the gland after hypothalamic destruction paralleled closely the diminution in circulating TSH levels. Thus a single thyrotrophin suffices to account for morphologic integrity of the thyroid as well as hormone release by the gland. The occasional necessity for postulating multiple thyrotrophins reflects inadequate knowledge of the primary actions of TSH on the sequential processes of thyroid hormone formation and release. On the basis of potentiating or inhibiting effects of various substances (for example, iodine) on

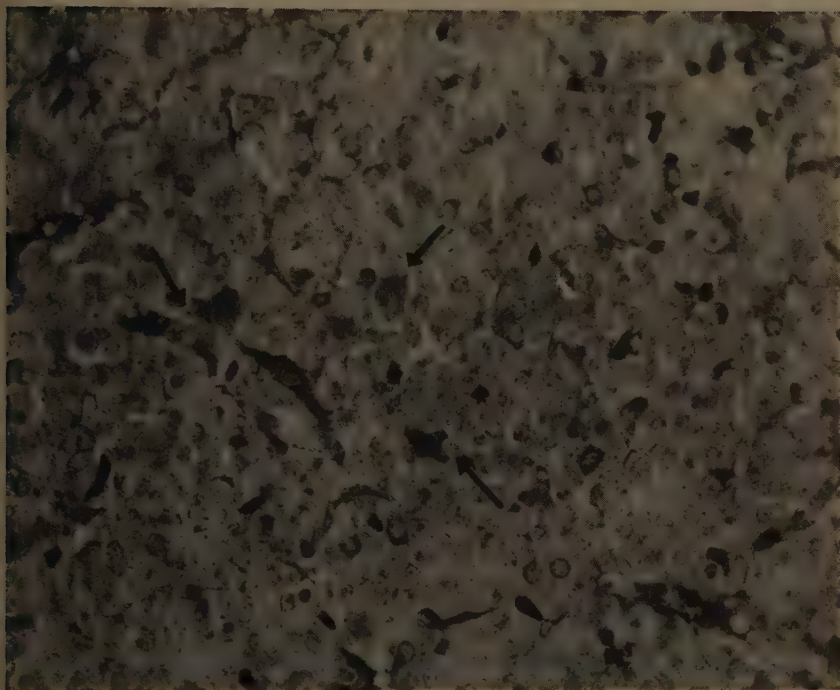


FIGURE 15. The persistence of large, deeply stained beta basophils (arrows), with almost complete disappearance of acidophils and delta basophils, in the *pars distalis* of an obese lesioned rat (Jm, TABLE 2). Masson. $\times 400$.

the response of thyroidal processes to TSH stimulation, we can explain apparent dichotomy in thyroid function without postulating more than one thyrotrophin.¹⁷

The accurate definition of TSH levels in the blood and pituitary of lesioned rats permits clearer interpretation of the role of the hypothalamus in the dynamics of thyrotrophic hormone secretion by the adenohypophysis. It is evident that an intact hypothalamus is required, not only for enhanced TSH secretion, as with goitrogens,⁷⁻¹² or partial thyroidectomy,¹³ but also for TSH secretion under normal demand conditions. Although assays of the pituitary do not reveal turnover rates of any trophic hormone, the reduction in total TSH stores in the gland at a time when blood levels are also decreased strongly suggests that after hypothalamic injury there is suppression of both synthesis and release of TSH from the pituitary. Nevertheless, it is clear that the rat's "isolated" pituitary has a good measure of autonomous TSH function after disruption of the hypothalamic link. Destruction of a considerable

portion of the hypothalamus did not prevent the massive discharge of pituitary TSH that characteristically follows the use of a goitrogen,^{12,17} nor did it prevent the reaccumulation of TSH in the pituitary after goitrogen withdrawal. The responses of the thyroid conformed closely; neither the histological changes nor the release curves approached those seen after hypophysectomy. Although it may be argued, even to the penultimate neuron, that some portion of the hypothalamus responsible for TSH regulation escaped injury, we consider this highly unlikely. The degree of hypothalamic destruction in this study appeared as great as, if not greater than, that described by others. More extensive lesioning would hardly have been compatible with survival. It should be emphasized that the residuum of TSH function, independent of hypothalamic influence, may be different in other species. The severity of morphologic and functional changes in the thyroid of the dog after hypothalamic lesioning¹⁴ indicates a more dominant role of the hypothalamus in TSH secretion for this species, as do certain experiments on the guinea pig (unpublished).

Classic views^{17,39,40} hold that the interaction between thyroid and anterior pituitary is a feedback or servomechanism in which the blood levels of their hormones regulate secretion of one another at the glandular source. The question as to whether thyroid hormone acts directly on the anterior pituitary to regulate TSH secretion or whether it does so via the hypothalamus has not been resolved by pituitary stalk section experiments. Interpretation of the results has been clouded by technical factors such as completeness of stalk division, the extent of hypophysial portal vessel regeneration, and generalized vascular damage to the adeno-hypophysis. Uotila^{5,41} concluded that intact hypothalamic-pituitary stalk connections were essential for augmentation of TSH secretion and thyroid hypertrophy to cold, but that disruption of the link did not interfere with a humorally controlled basic TSH secretory rhythm. Brolin^{42,43} maintained that hypothalamic-pituitary stalk continuity was required for normal thyrotrophic tonus as well. The ensemble of results by Barnett and Greep⁴⁴⁻⁴⁶ led them to conclude that hypofunction of the pituitary and target-gland atrophy after section of the stalk resulted primarily from a generalized vascular insult to the adeno-hypophysis and not from deprivation of either neural or neurohumoral influences. Some increase in TSH and adrenocorticotrophic hormone (ACTH) secretion could be induced in cold, exposed, stalk-sectioned rats despite lack of neural or portal continuity between pituitary and hypothalamus.⁴⁶ Harris² insisted that partial regeneration of the hypophysial portal vessels may have occurred in these experiments.

The present study presents direct evidence to the effect that thyroid hormone acts directly on the adeno-hypophysis independently of the hypothalamus, and that the servomechanism between thyroid and anterior

pituitary is not under neural domination. Triiodothyronine and thyroxine markedly suppressed TSH secretion in the pituitary of the lesioned rat. The inhibition was not quantitatively different from that in the intact animal. The fact that triiodothyronine was more potent than thyroxine is in accord with previous studies indicating a similar relationship for other parameters of thyroid function in the rat.⁴⁷⁻⁴⁹ There is strong indication that functional integrity of the thyroid-pituitary servomechanism is preserved in the rabbit also, despite disruption of the hypothalamic-pituitary link. Utilizing ingenious microtechniques, von Euler and Holmgren²⁰ found that local injection of thyroxine into the anterior pituitary of the conscious rabbit inhibited thyroidal I^{131} release; the effect was not elicited with injections into the hypothalamus or median eminence. Similarly, thyroidal I^{131} release in hypophysectomized rabbits bearing adenohypophysial transplants in the anterior chamber of the eye also could be inhibited by raising the blood concentration of thyroxine.²¹ Results of pituitary stalk-section experiments in the rabbit (Brown-Grant, Harris and Reichlin; see Harris²) point in the same direction. Complete division of the stalk reduced thyroid activity, as measured by release rate, to levels slightly higher than in the hypophysectomized animal. Inhibition of thyroidal I^{131} discharge could be accomplished with thyroxine (or laparotomy), although the thyroid inhibition usually forthcoming with restraint or stilbestrol injection was abolished. Inasmuch as these rabbit studies were done without benefit of bioassay, strict comparison with results of this study cannot be made. Thyroidal radioiodine release does accurately reflect the tempo of thyroid secretion, but it does not necessarily give an accurate measure of TSH secretion by the pituitary. In the above experiments, thyroidal I^{131} release rate in hypophysectomized rabbits bearing pituitary grafts was essentially normal, yet the release rate was markedly reduced with pituitary stalk section. Furthermore, the relative effectiveness of triiodothyronine and thyroxine on inhibition of thyroidal I^{131} release in the intact rabbit was of the order of 1.3:1.⁵⁰ Suppression of TSH secretion in the rat, as measured by assay, was about 5:1.

The experimental dissociation of the pituitary-thyroid system from the hypothalamus affords a clearer view of the role of the latter in the regulation of TSH secretion. The hypothalamus functions as a modulator; it modifies the production and release of TSH from the adenohypophysis, not only under conditions of increased demand, but also in meeting normal day-to-day requirements. In the absence of the hypothalamic stimulus, normal blood levels of TSH cannot be maintained. The anterior pituitary, however, retains its capacity to produce and release TSH, subject only to regulation imposed on it by a systemic stimulus, namely, the level of circulating thyroid hormone. The action of thyroid hormone

in this system occurs directly at the anterior pituitary. The general failure to find selective concentration of labeled thyroid hormones in the adenohypophysis, in contrast to the neurohypophysis, does not contradict this.^{18,19,51} A hormone need not be concentrated at its locus of activity to exert its effects; TSH induces profound effects at the thyroid without evidence for selective concentration there.⁵² The localization of labeled thyroid hormones in the neurohypophysis may signify a special action on this neural mechanism, possibly in relation to neurosecretory processes in the posterior pituitary. Regulation of TSH secretion by the posterior pituitary appears doubtful from the present study. TSH activity in lesioned animals bore no apparent relationship to the functional state of the neurohypophysis. Reduced thyrotrophic hormone secretion occurred with or without atrophy of *pars nervosa* or damage to the paraventricular nucleus; moreover, thyroxine inhibited TSH secretion in the adenohypophyses of lesioned rats with diabetes insipidus. The precise nature of the hypothalamic stimulus for TSH regulation cannot be stated from this or previous work. No specific nucleus or fiber pathway in the hypothalamus has been implicated, nor has any neurohumor been identified. Brown-Grant⁵³ has speculated that the role of the hypothalamus in the control of TSH secretion is to prevent exposure of the anterior pituitary to the normally suppressive influence of circulating thyroid hormone. This would be accomplished by selective removal of thyroid hormone from the arterial blood during its passage through the primary plexus of the hypophysial portal system. The logical extension of this concept to other target hormones would require that the hypothalamus be a "filtering" mechanism par excellence. Rate, as well as content, of blood flow through the pituitary may be important. Neither has been measured. Peripheral utilization and inactivation of both thyroid and thyrotrophic hormones are also factors that must be considered.

The histochemical studies of Halmi^{33,34} and Purves and Griesbach^{31,35,36} on the rat hypophysis have indicated that the broad category of basophils, selectively stained with aniline blue in Mallory or Azan techniques, consists of at least two functionally distinct types differing in morphology, topographical distribution, and reactivity to glycoprotein stains. Aside from minor disagreement regarding the distribution of these cell types (beta and delta cells were frequently found in close proximity in ventral regions of the anterior lobe), the structural changes in the adenohypophysis of lesioned rats are in conformity with the interpretations of Halmi and Purves and Griesbach that the angular, PAS, and aldehyde-fuchsin-staining basophils elaborate the thyrotrophic hormone. All cell types in the adenohypophysis were affected after hypothalamic destruction, but the beta cells persisted even with the

most extensive lesion, and their presence could be correlated with maintenance of normal TSH concentrations in the gland. Conversely, in pituitaries depleted of TSH by exogenous thyroid hormone, there was almost complete disappearance of the thyrotrophs. The delta basophils responded more sensitively to hypothalamic lesioning; the marked reduction in numbers and cytological activity of this cell type correlated well with ovarian atrophy. Delta cells reappeared in substantial numbers with thyroid administration. The significance of the rise in numbers and cytological activity of the delta basophils after thyroid hormone treatment is not clear, especially since the ovaries remained atrophic; gonadotrophic and ACTH assays of the pituitary are clearly indicated.

The selective impairment in target-gland morphology after hypothalamic lesioning and the differential response of the glands to exogenous thyroid hormone indicate discriminative effects on pituitary trophic hormone secretion. It is difficult to substantiate the alternative view that the target-gland differences after hypothalamic destruction are merely reflections of sensitivity to loss in a single integrative neuroendocrine mechanism. It was found possible by variation in lesion placement to curtail TSH secretion with little or no apparent change in the pituitary-ovarian system. In fractional hypophysectomy, the reverse is usually the case.^{54,55} Extensive lesions, while affecting thyroid and ovarian function, produced adrenal atrophy infrequently, and could not prevent appreciable adrenal hypertrophy with thyroid hormone treatment. Greer and Erwin⁹ also found evidence of increased ACTH secretion with simultaneous decrease in TSH activity in lesioned rats receiving "amphenone" (a synthetic compound with stimulating effects on ACTH and TSH secretion). These results do not negate a large body of evidence that implicates the hypothalamus in the regulation of ACTH release from the pituitary.⁵⁶⁻⁵⁸ They more plausibly indicate that augmented ACTH secretion in the pituitary can occur under some conditions of chronic stress without hypothalamic intervention. Laqueur *et al.*⁵⁹ have reached similar conclusions for the cat. As in earlier investigations, the present study demonstrates the frequent association between obesity and genital atrophy after hypothalamic damage.^{60,61} Histochemical evaluation of the basophil cell picture in the adenohypophyses of lesioned rats leaves little doubt that morphologic regression of the female reproductive tract was referable to a primary disturbance in gonadotrophin secretion. The degree of ovarian atrophy after hypothalamic destruction appeared to be greater than that described in earlier studies on the rat,^{15,62} possibly because of more frequent damage to the median eminence and arcuate nucleus. Lesions in this area in the male rat correlate best with decreased gonadotrophic potency of the pituitary.¹⁶ In the absence of data on cyclic behavior, and without gonadotrophic assays,

it cannot be stated to what extent follicle-stimulating hormone and luteinizing hormone secretion were differentially affected.

Summary

Electrolytic lesions in the anterior and tuberal portions of the hypothalamus of the female rat selectively disturbed trophic hormone secretion in the adenohipophysis with corresponding effects on the target endocrines. Thyroid activity, judged by histology and I^{131} release from the gland, was reduced after hypothalamic destruction. Inhibition of thyroidal radioiodine release occurred as early as two weeks after lesioning and was more marked in rats that became obese. A significant reduction in the titers of circulating TSH also was found. The close parallelism between the morphologic and release changes in the thyroid and the blood levels of TSH does not lend support to the concept of dual thyrotrophins. TSH concentration in the pituitaries of lesioned rats was normal, but total stores of hormone decreased. The administration of thyroxine and triiodothyronine markedly suppressed TSH secretion in the adenohipophyses of lesioned rats; triiodothyronine was more potent (approximately 5:1) in this respect.

The physiological and histochemical alterations induced in the adenohipophysis by electrocautery strongly implicated the basophil (beta cell) in the genesis of thyrotrophic hormone. All anterior pituitary cell types were affected by lesioning, but only the beta cell persisted when TSH stores were high or, conversely, disappeared when the pituitary was depleted of TSH with exogenous thyroid hormone. Hypothalamic lesioning did not prevent the reaccumulation of TSH in pituitaries previously depleted of their hormone by propylthiouracil; pituitary TSH concentrations rebounded to supernormal levels, but titers in blood decreased to subnormal values. The maintenance of abundant TSH reserves in the adenohipophysis, their depletion with thyroid hormone, and the reaccumulation of TSH in the gland after goitrogen withdrawal indicate (1) that a good measure of autonomous TSH function exists in the "isolated" pituitary, (2) that thyroid hormone can act directly on the adenohipophysis independently of the hypothalamus, and (3) that the basic servomechanism between thyroid and anterior pituitary is not under neural domination.

Extensive damage to the hypothalamus did not affect the target endocrines with equal severity. Basal lesions in the anterior hypothalamus, that included damage to the arcuate nucleus and median eminence, usually resulted in obesity, ovarian atrophy, and reduced thyroid function. Paramedian lesions in the dorsal hypothalamus diminished thyroid activity without morphologic change in the ovary. Adrenal atrophy

was not consistently produced even after marked hypothalamic damage. Thyroid hormone administration to lesioned rats induced adrenocortical hypertrophy, but failed to correct ovarian atrophy.

Conclusions

The role of the hypothalamus in the hypothalamic-hypophyseal-thyroid system is that of a modulator; it effects quantitative adjustments in the system by modifying production and release of TSH from the basophils (beta cells). This regulatory influence on TSH secretion exists for normal as well as for emergency conditions in which augmentation of TSH secretion is required. The basic interplay between thyroid and anterior pituitary, however, is not under neural domination, but is systemically controlled by the level of circulating thyroid hormone. In this system, thyroid hormone suppresses TSH secretion at the adeno-hypophysis without hypothalamic mediation.

The cytophysiological changes in the adeno-hypophyses of hypothalamically lesioned rats, the selective impairment in target-gland morphology, and the differential response of these glands to exogenous thyroid strongly imply the presence of individual and discriminative hypothalamic-hypophyseal mechanisms in the regulation of trophic hormone secretion.

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